



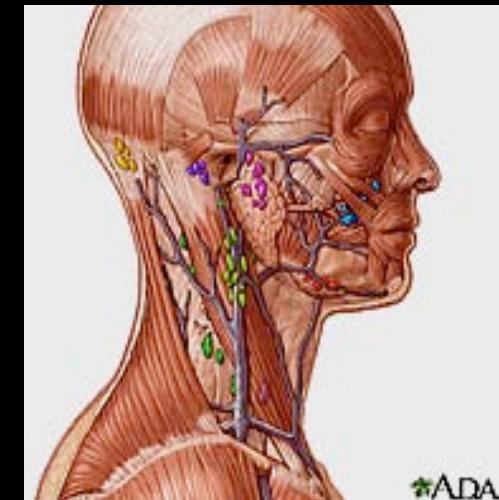
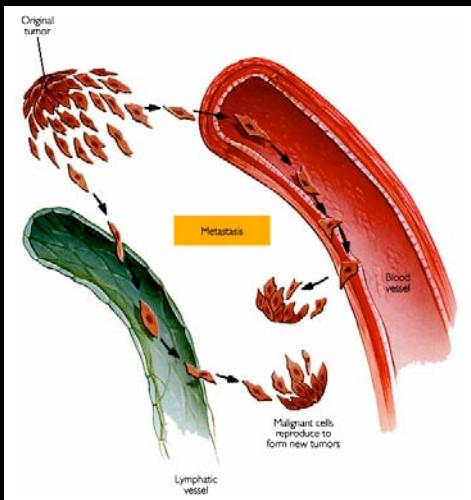
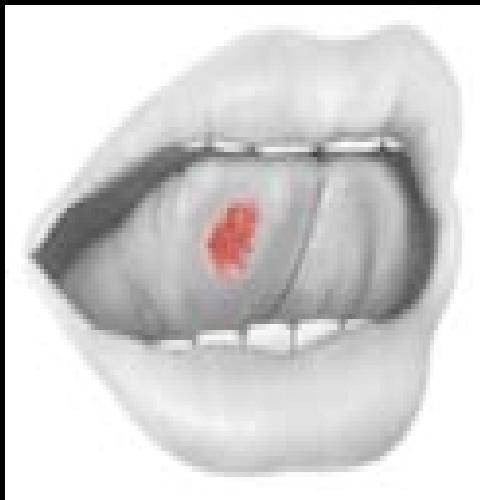
A Molecular Portrait of Head and Neck Cancer Progression

Vyomesh Patel

Oral and Pharyngeal Cancer Branch
National Institute of Dental and Craniofacial Research
National Institutes of Health, DHHS

Tongue SCC

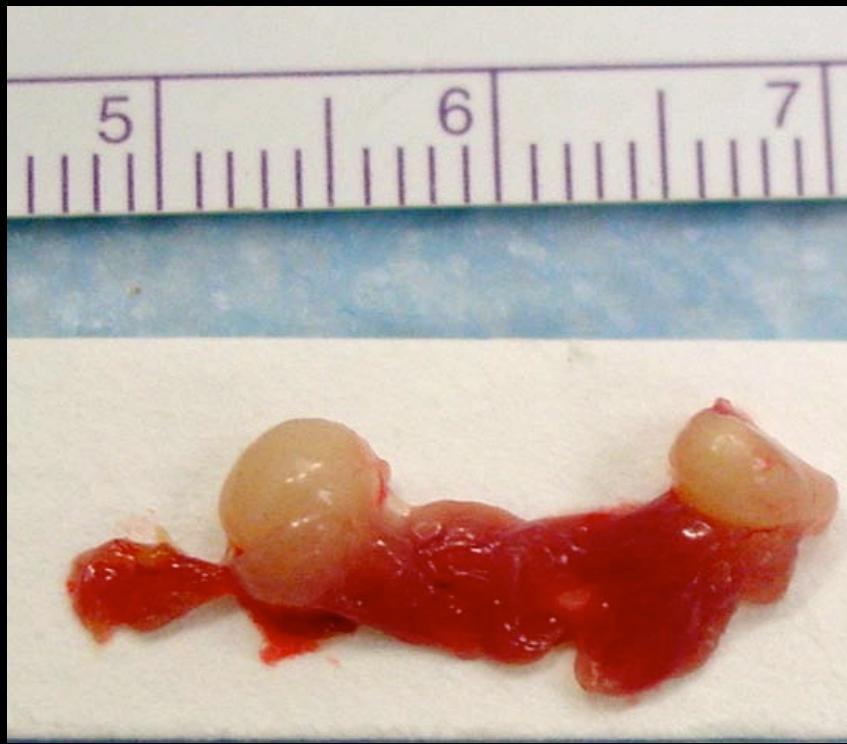




→
cancer
development

→
cancer
progression

Decreased Survival



Understanding Head and Neck Cancer Pathogenesis



Betel and areca nut

Viral infection (HPV, EBV, other)

Occupational environment (dust, coal tar, asbestos)

Food component (nitrite and nitrosamine consumption)

Dental factors (chronic ulcer, poor oral hygiene/denture)

Head and Neck Cancer

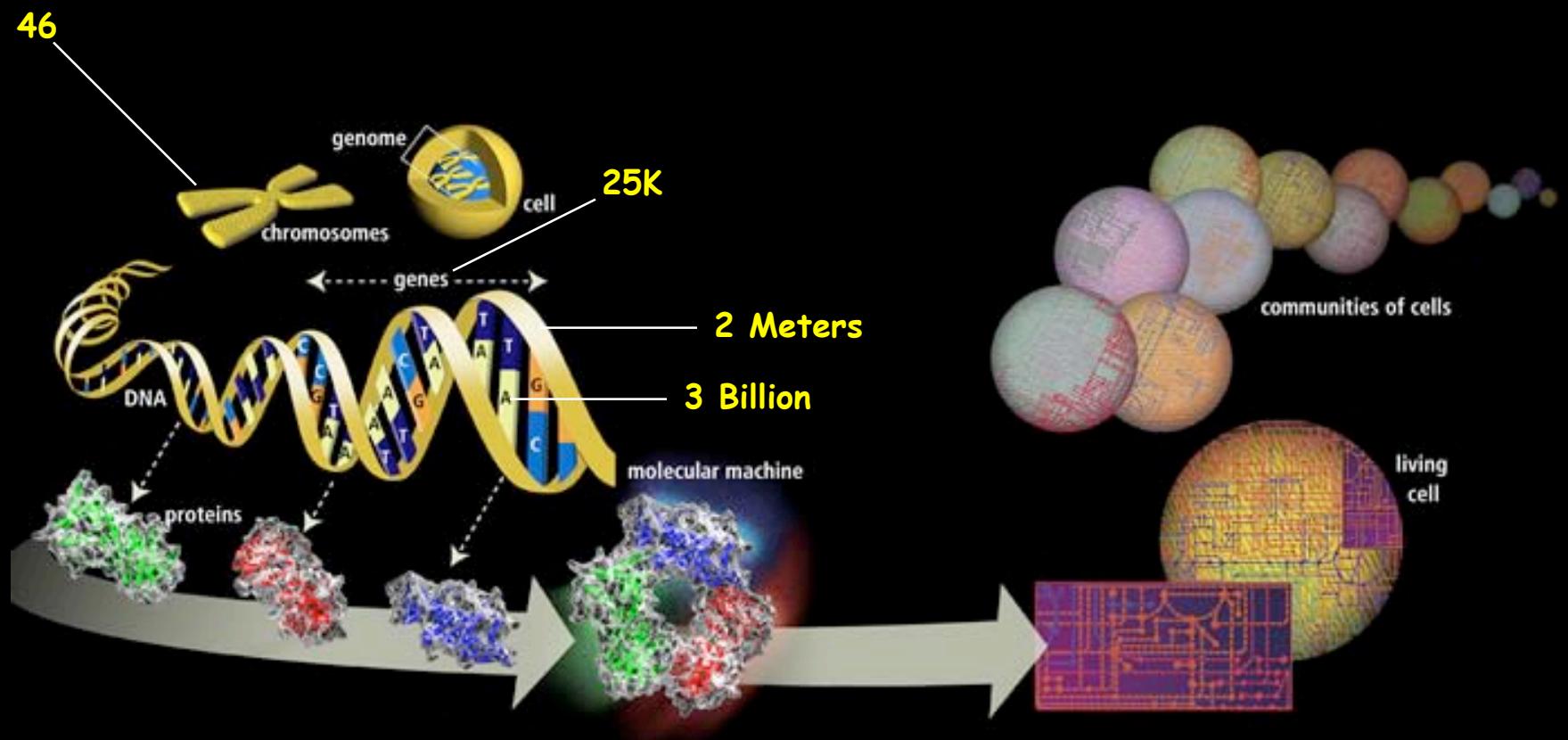
The many **EFFECTS** of **ORAL CANCER**

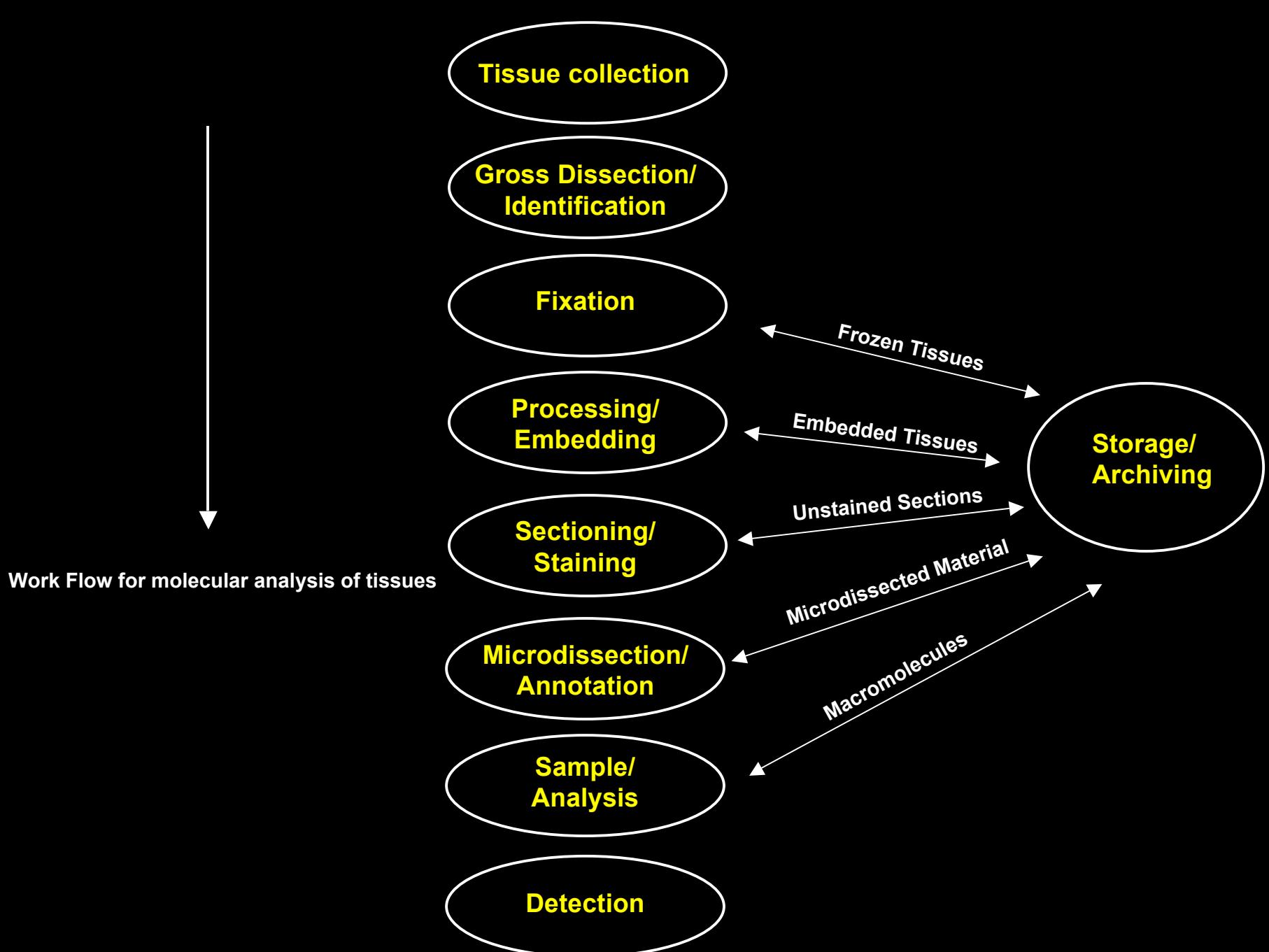


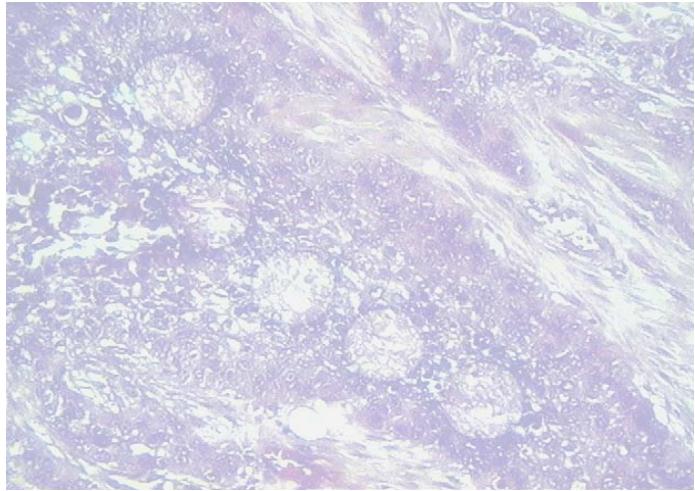
Get checked before it's too late!
IT'S PAINLESS & FREE.

1.877.7 CHECKED

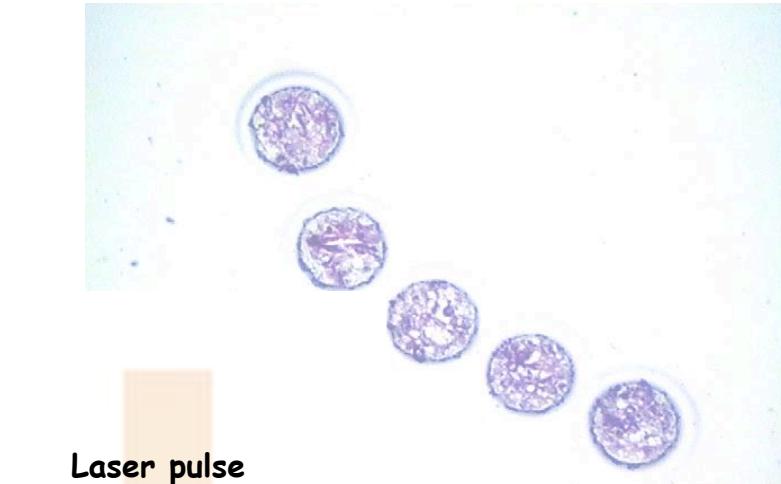
Molecular basis of this cancer still unclear





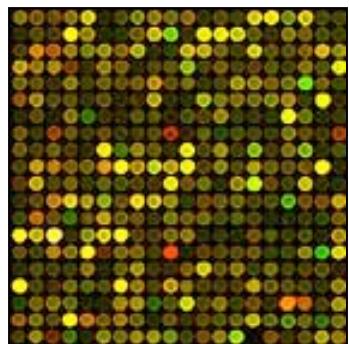


Laser Capture Microdissection

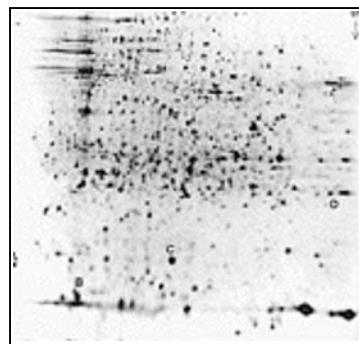


Laser pulse

Cells of interest



Expression analysis
(Arrays, proteomics)
CGH

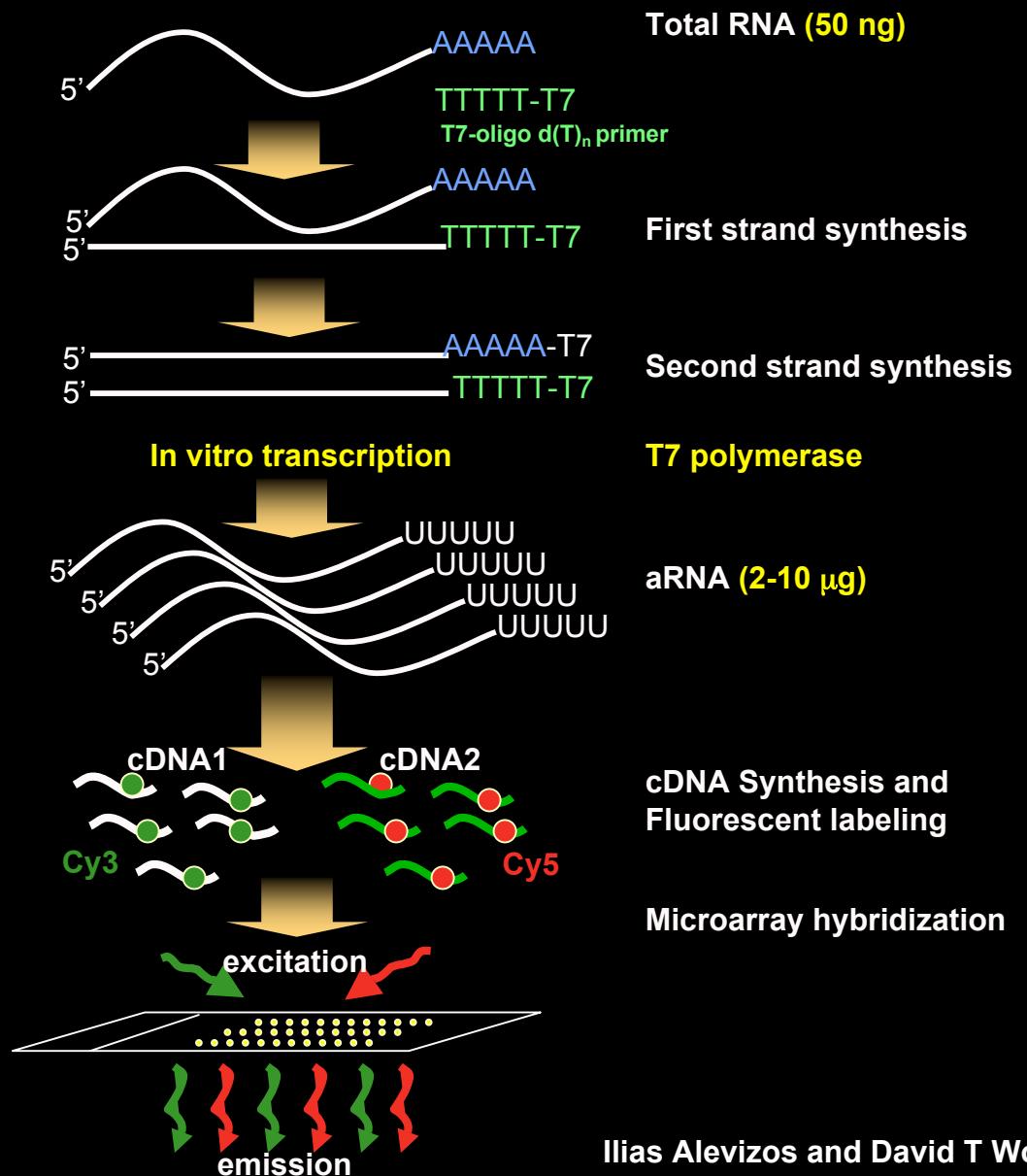
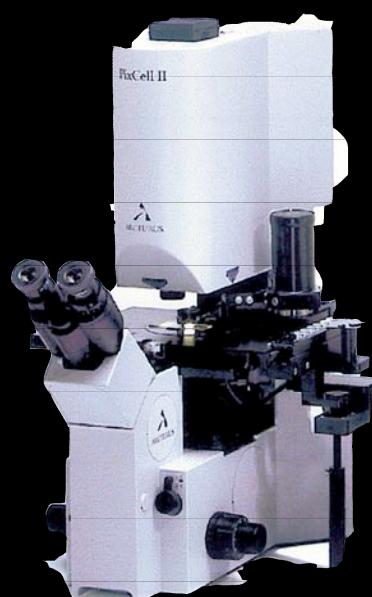
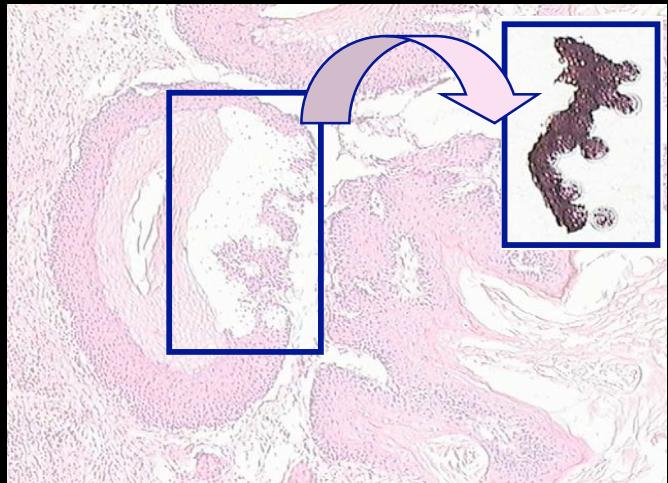


Extraction
RNA, DNA, Protein



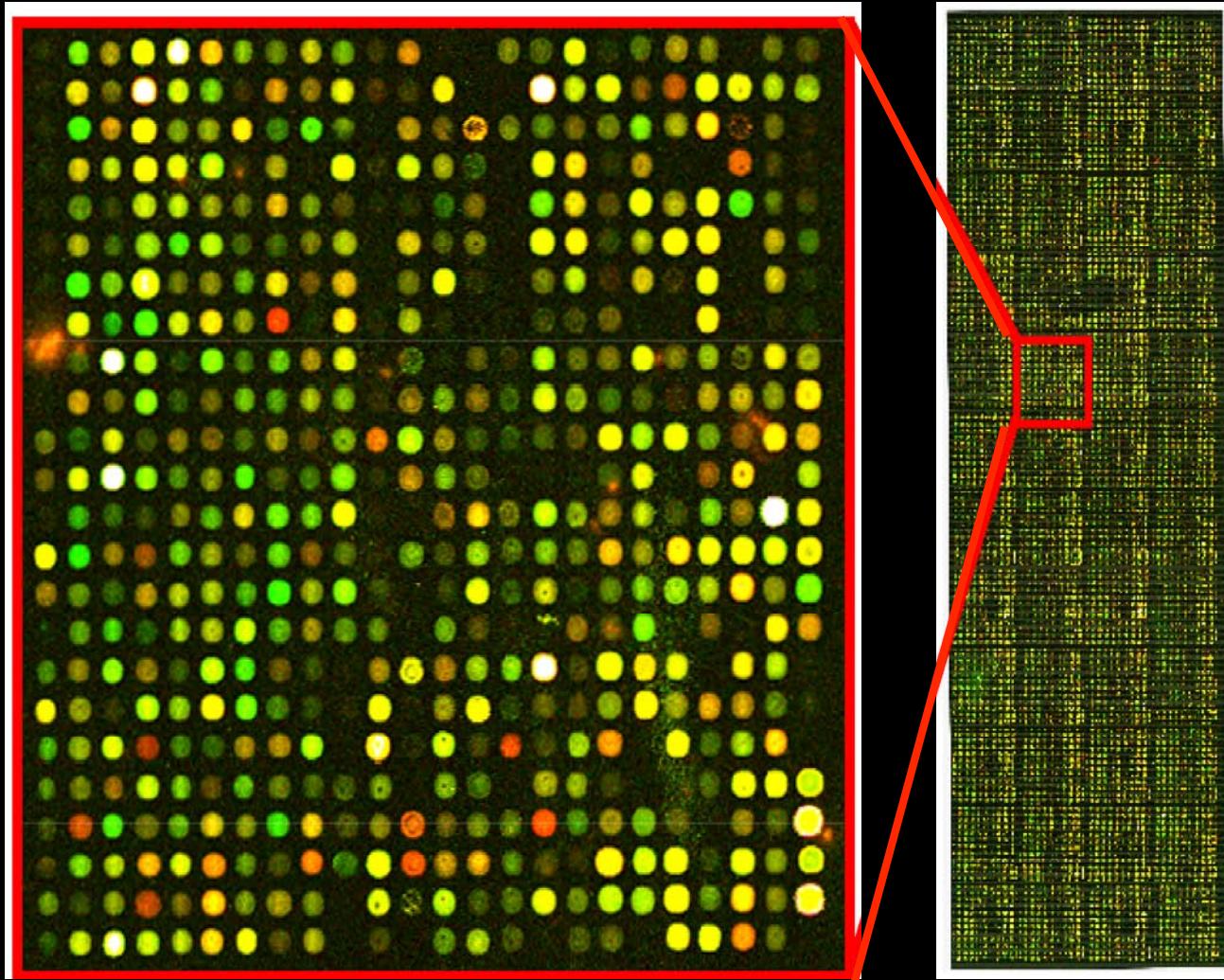
Lysis
buffer

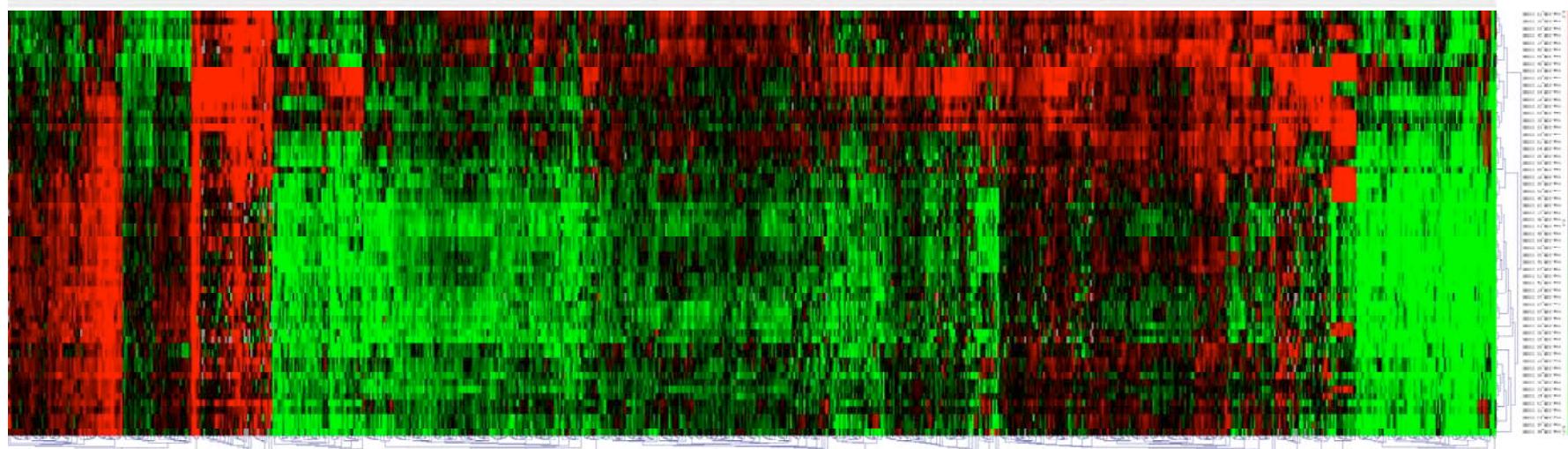
T7 amplification and cDNA microarrays



Ilias Alevizos and David T Wong

Whole Genome on Slide now Available





Gene	R,C	log2 Normal	log2 Tumor	log2 (tumor)	10% FDR	5% FDR	1% FDR
PTHLH	199,79	-0.46469	5.094223	5.55891	yes	yes	yes
MMP10	75,56	-1.8804	3.538677	5.419076	yes	yes	yes
MMP1	14,20	-4.24605	0.916526	5.162579	yes	yes	yes
POPD3C	150,42	-4.84179	-0.15025	4.69154	yes	yes	yes
HOXA1	61,26	-3.49337	1.095443	4.588816	yes	yes	yes
PHLDB2	153,23	-2.32848	2.26008	4.58856	yes	yes	yes
PTHLH	87,79	0.597757	5.12967	4.531913	yes	yes	yes
MMP10	19,56	1.080286	5.429114	4.348827	yes	yes	yes
HMGAA2	182,20	-6.23674	-2.0125	4.224234	yes	yes	yes
SAA1	21,21	0.372686	4.347987	3.975301	yes	yes	yes
MFAP5	286,52	-1.76517	2.181735	3.946903	yes	yes	yes
WDR66	44,10	1.936286	5.81048	3.874195	yes	yes	yes
Unknown	27,19	-0.11866	3.745802	3.864459	yes	yes	yes
AIM2	45,18	0.459758	4.318317	3.858559	yes	yes	yes
SAA3P	242,107	-1.17056	2.675743	3.846303	yes	yes	yes
TGFBI	232,14	-2.72184	1.09529	3.817129	yes	yes	yes
IL24	101,89	-0.08641	3.712763	3.799169	yes	yes	yes
LAMC2	30,12	1.695341	5.488223	3.792881	yes	yes	yes
SERPINE1	230,28	-2.66128	1.098679	3.759962	yes	yes	yes
PDPN	297,59	-2.99739	0.709239	3.706627	yes	yes	yes
IL8	76,58	-5.45185	-1.74795	3.703894	yes	yes	yes
MAGEA2B	70,36	-4.96638	-1.29966	3.666726	yes	yes	yes
INHBA	120,76	-1.60043	2.046821	3.647251	yes	yes	yes
PHLDB2	41,23	-0.95054	2.682331	3.632871	yes	yes	yes
ISG15	17,62	-1.96554	1.666235	3.631773	yes	yes	yes
ADAM12	238,96	-5.09481	-1.46436	3.630451	yes	yes	yes
MMP3	190,42	-3.84891	-0.25183	3.59708	yes	yes	yes

Why Proteins?

- Protein expression levels are not predictable from mRNA expression levels.
- Proteins are uniquely modified and processed in ways which are not apparent from the gene sequence
- Proteins are dynamic and reflect the state of biological systems.

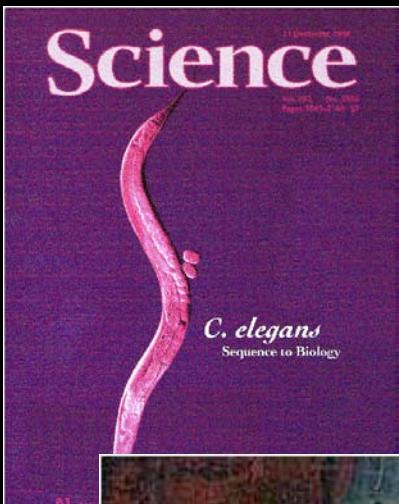


Storage of freshly frozen biopsies

Genomics

1953: Watson and Crick: DNA double helix

C. Elegans



Rattus norvegicus



Danio rerio



Anopheles gambiae



Fugu rubripes



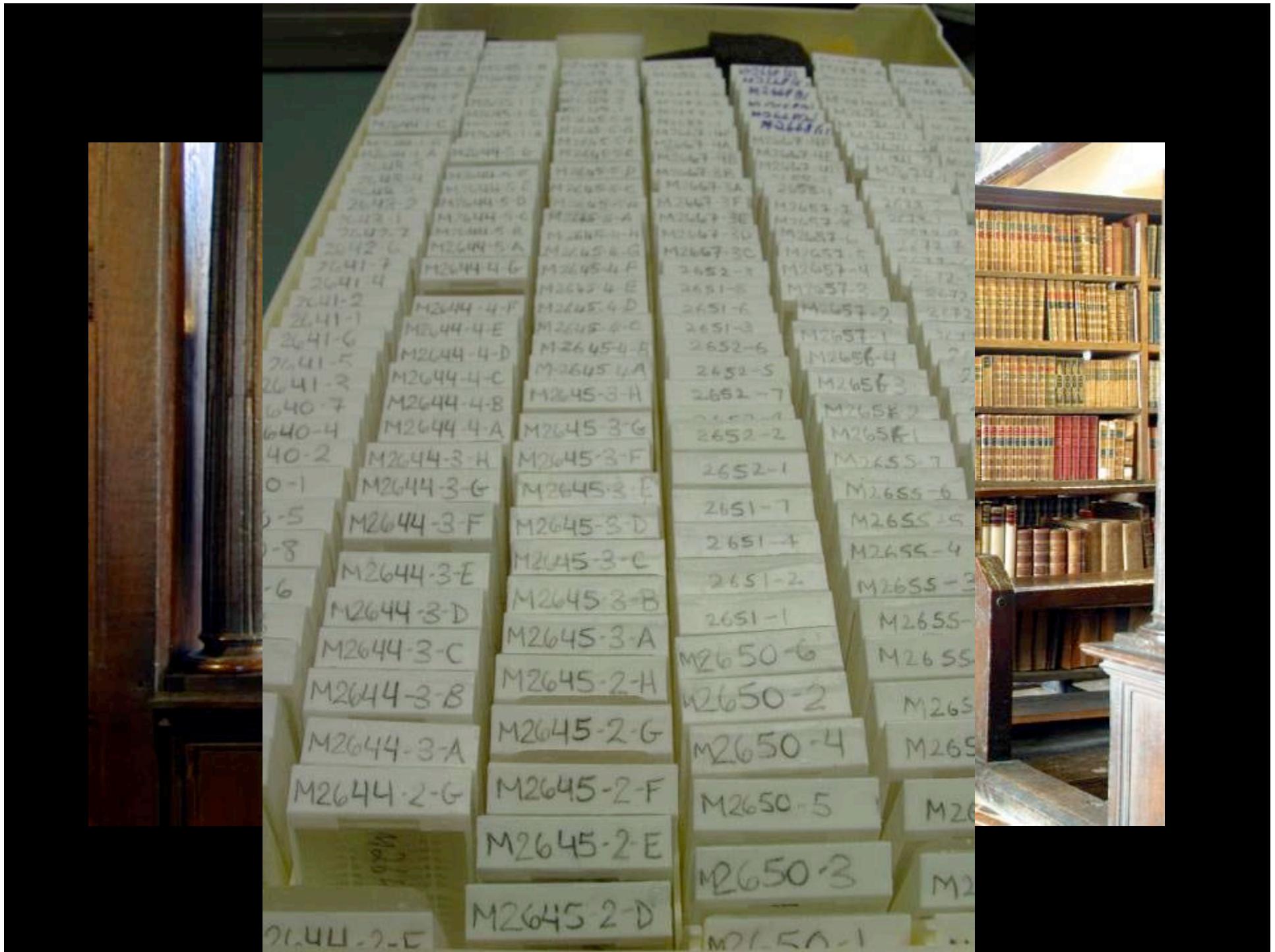
Drosophila melanogaster



Homo sapiens



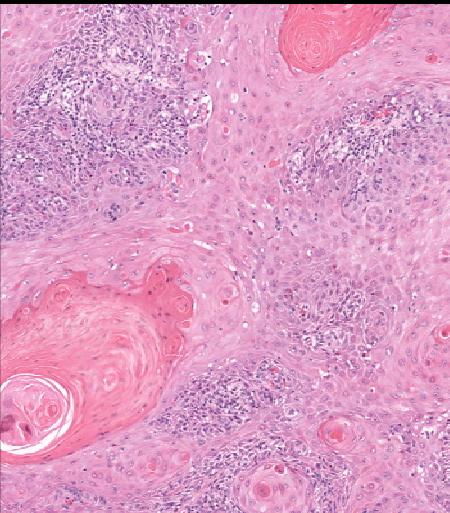
Mus musculus



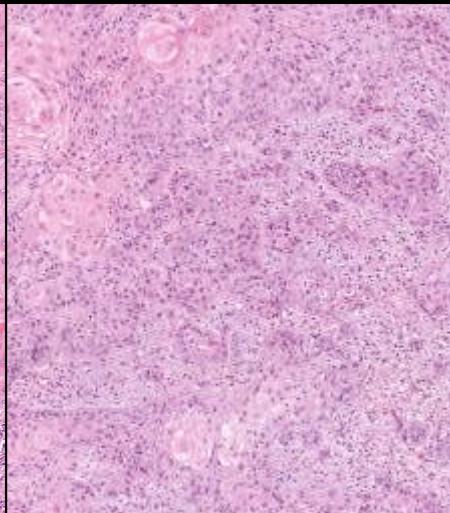
Non-Malignant



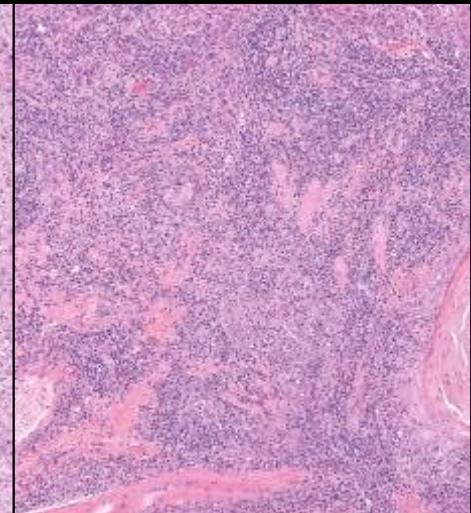
WD



MD



PD



WD: Well Differentiated

MD: Mild Differentiated

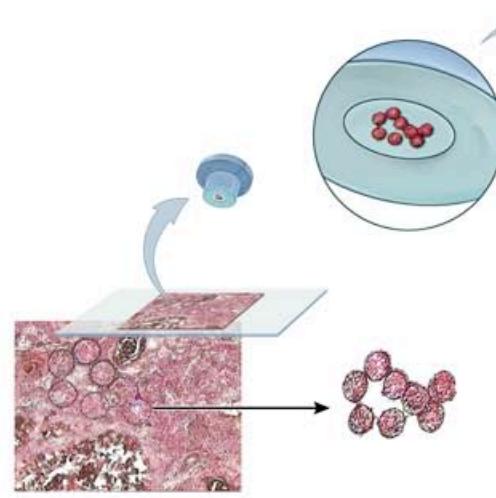
PD : Poorly Differentiated

Unlike FFPE, fresh frozen tissue not readily available

Massive retrospective study in clinically relevant cases possible

Excellent morphology

Work Flow

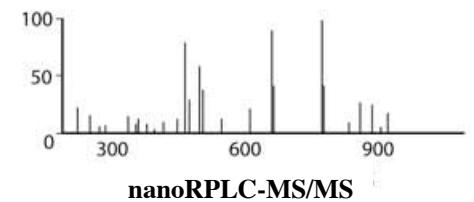
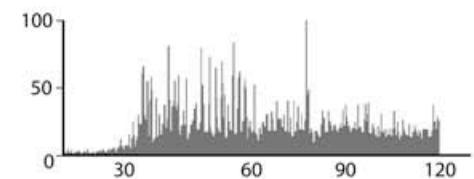


LiquidTissue™

Trypsin and Tryptic Peptides

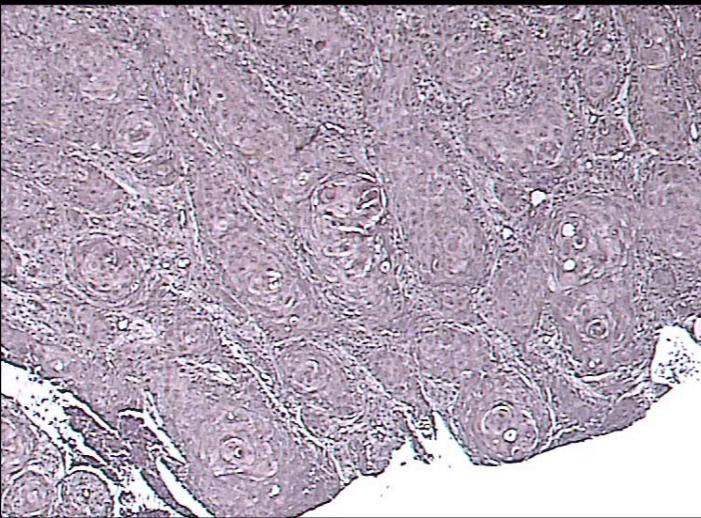
DTT

Sample Cleanup

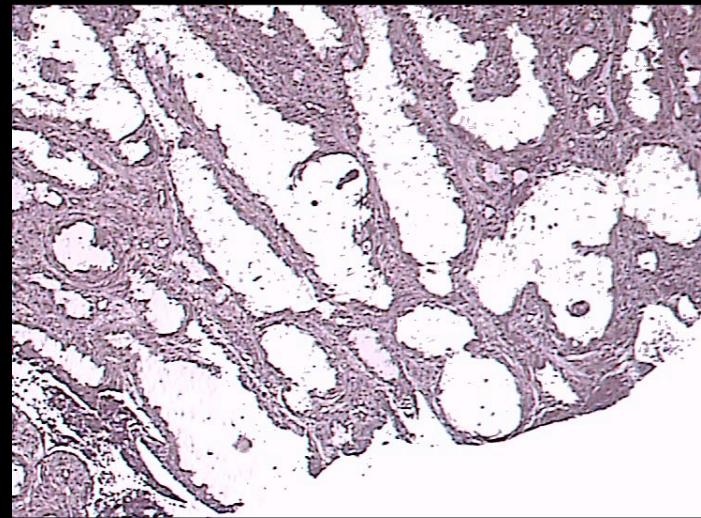


nanoRPLC-MS/MS

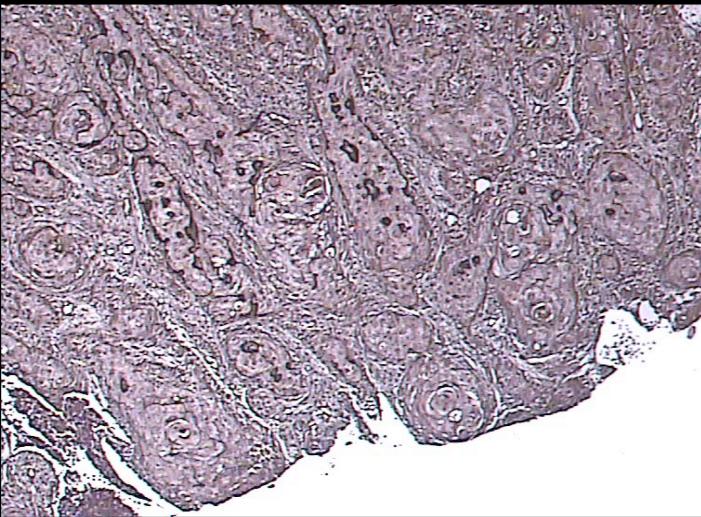
WD HNSCC



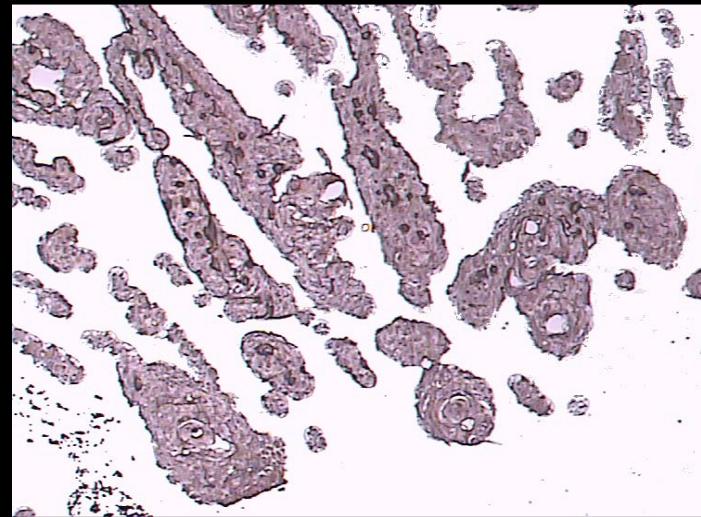
Before



After



Laser

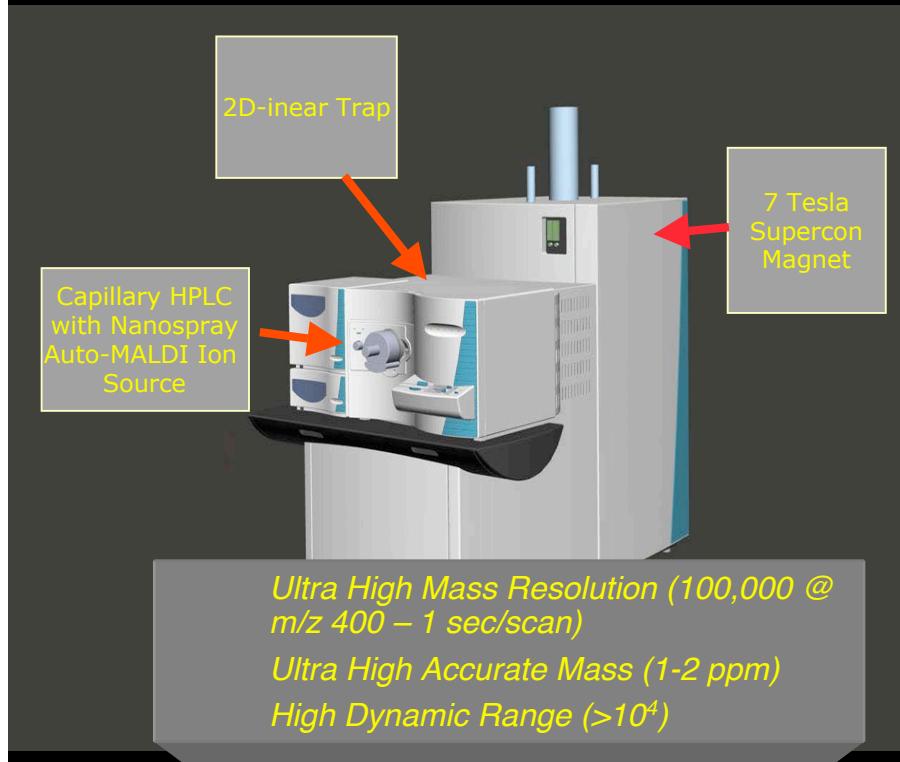


Laser Capture Microdissection

Caps:
Extract protein

Linear Trap-FTMS

(Electrospray ionization-Fourier transform ion cyclotron resonance mass spectrometry)



Pathology	Yield (μg)	Unique Proteins	> 2 peps	Total (all 4)
Norm	< 1.06	207	30	
Norm	< 1.06	140	23	
Norm	< 1.06	137	14	
Norm	< 1.06	106	14	391
Poor	2.72	239	45	
Poor	1.70	296	60	
Poor	< 1.06	257	46	
Poor	< 1.06	185	38	676
Moderate	4.46	366	71	
Moderate	3.95	322	65	
Moderate	2.04	216	34	
Moderate	1.11	194	29	729
Well	2.28	376	62	
Well	1.53	356	62	
Well	1.60	351	68	
Well	3.38	323	50	866

Data indicates trends in expressed proteins correlating with differentiation

Validation of data in progress

Scope for discovery

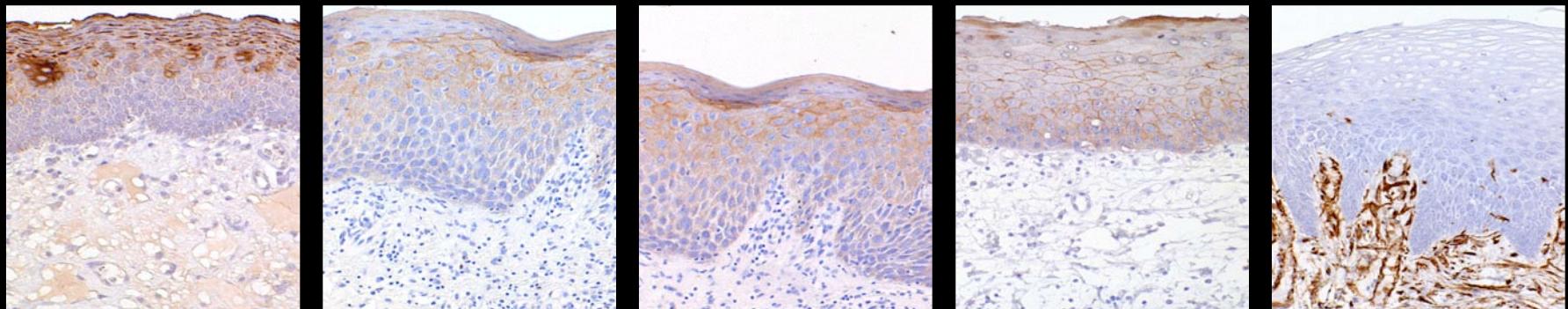
FFPE
20K cells

Representative proteins identified as potential biomarkers for HNSCC

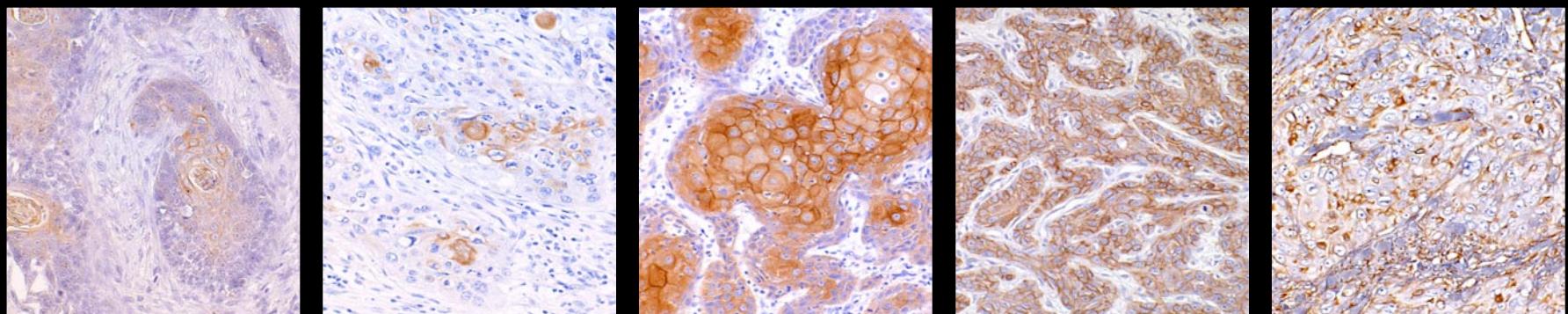
Accession	Protein	Peptides (number)	Normal (%)	Well D (%)	Mod D (%)	Poorly D (%)
P02533	Keratin, type I cytoskeletal 14	449	7.8	42.54	29.18	20.49
P08779	Keratin, type I cytoskeletal 16	387	8.27	41.09	30.23	20.41
P19013	Keratin, type II cytoskeletal 4	179	77.65	3.91	12.29	6.15
P04264	Keratin, type II cytoskeletal 1	146	18.49	22.6	28.77	30.14
Q04695	Keratin, type I cytoskeletal 17	76	6.58	40.79	31.58	21.05
P05783	Keratin, type I cytoskeletal 18	13	0	23.08	15.38	61.54
P08729	Keratin, type II cytoskeletal 7	11	0	0	90.9	10.1
P15924	Desmoplakin (DP)	385	15.32	43.9	18.44	22.34
Q13835	Plakophilin 1 (Band-6-protein)	95	16.84	30.53	21.05	31.58
P32926	Desmoglein 3 precursor	65	15.38	29.23	30.77	24.62
Q9Y446	Plakophilin 3	35	28.57	28.57	31.43	11.43
Q02487	Desmocollin 2A/2B precursor (Desmocollin-3)	7	0	42.86	28.57	28.57
Q76E58	Epiplakin	1	0	0	100	0
Q14764	Major vault protein (MVP)	18	5.56	33.33	38.89	22.22
P07476	Involucrin	57	8.77	56.14	26.32	8.77
P08670	Vimentin	79	0	36.71	44.3	18.99
P16144	Integrin beta-4 precursor (GP150) (CD104)	4	0	0	25	75
P09211	Glutathione S-transferase P (GSTP1-1)	22	4.55	31.82	22.73	40.91
P04792	Heat-shock protein beta-1 (HspB1) (HSP 27)	83	4.82	28.92	31.33	34.94
P11142	Heat shock cognate 71 kDa protein	20	5	30	25	40
Q86U54	CD151 antigen (CD151 protein)	1	0	0	100	0
O43490	Prominin 1 precursor (CD133 antigen)	1	0	0	0	100

Validation

Normal



Tumor



Cytokeratin 4

Cytokeratin 16

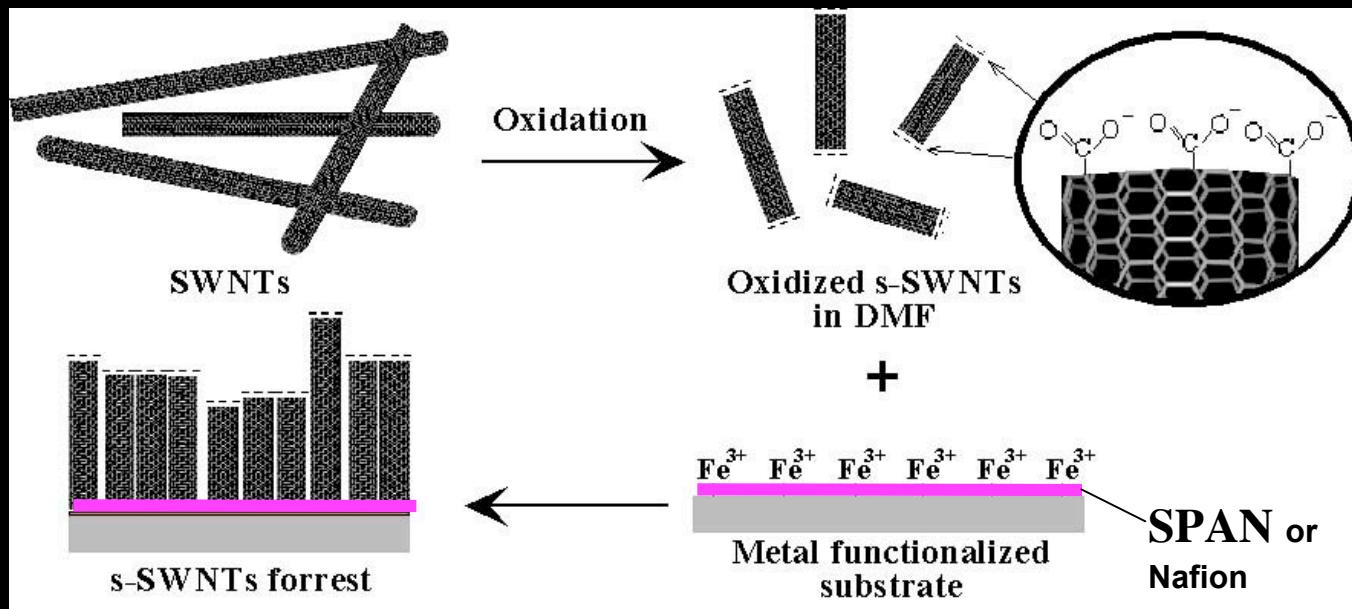
Desmoplakin

Desmoglein 3

Vimentin

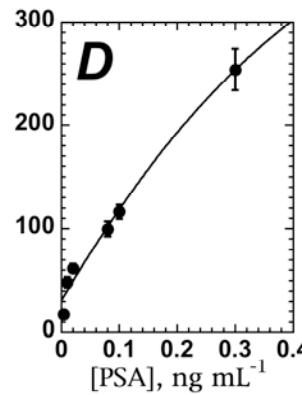
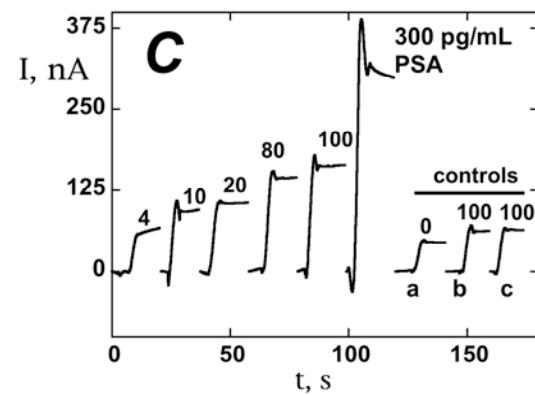
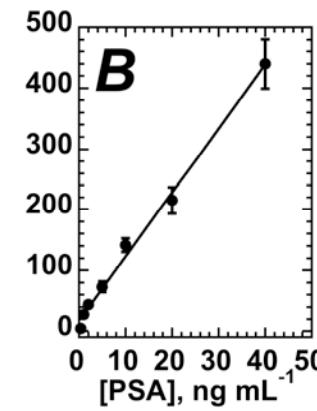
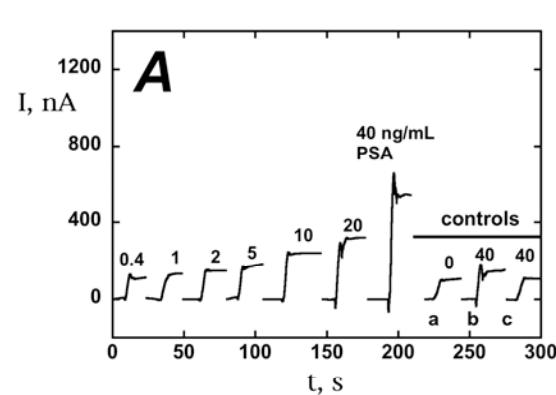
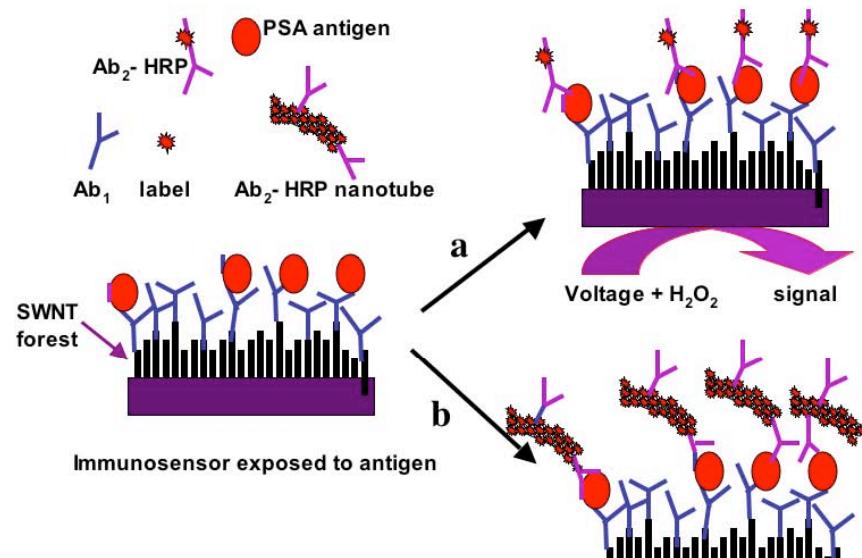
Single-Walled Carbon Nanotube Forests: Antigen-Antibody Sensing

~1.4 nm diameter, high conductivity

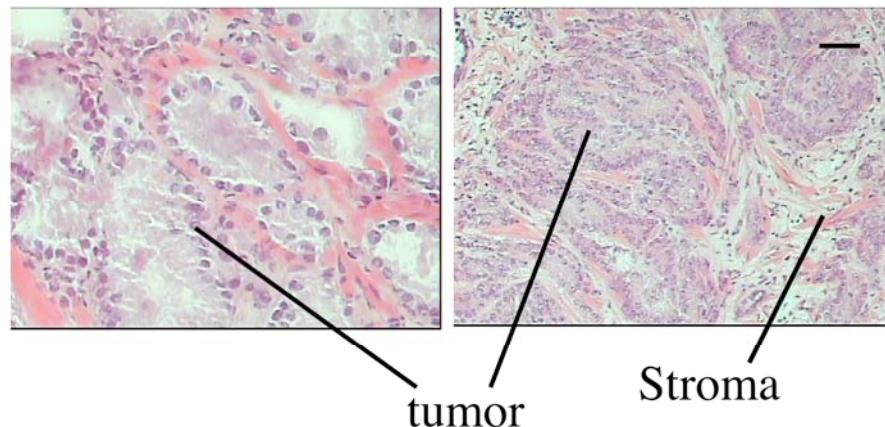


End COOH groups allow chemical attachment to proteins (antibodies)

High conductivity to conduct signal (e's) from enzyme label to meas. circuit

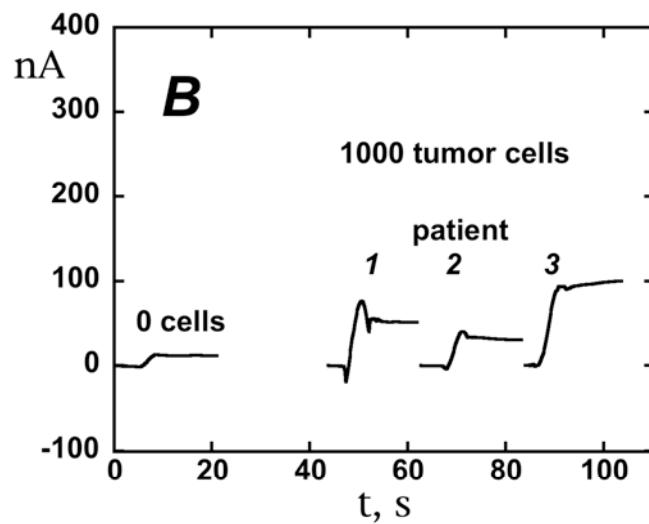


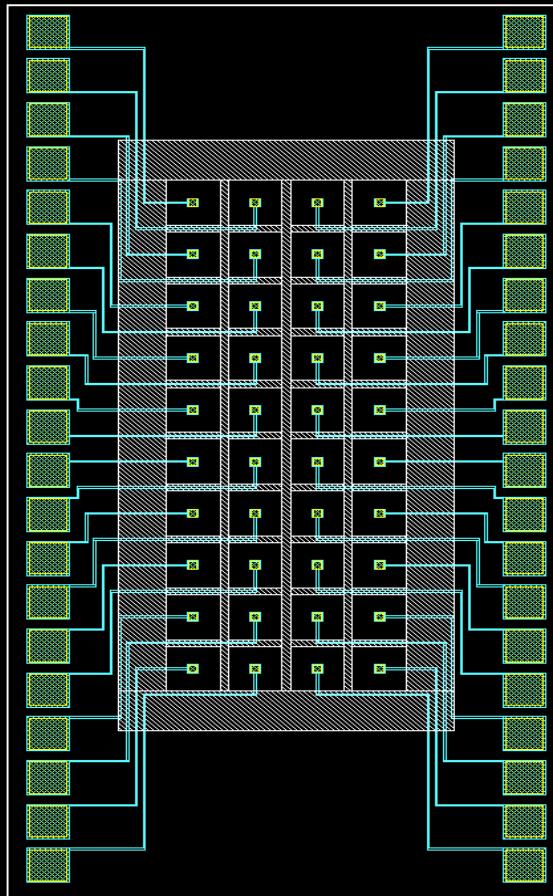
A



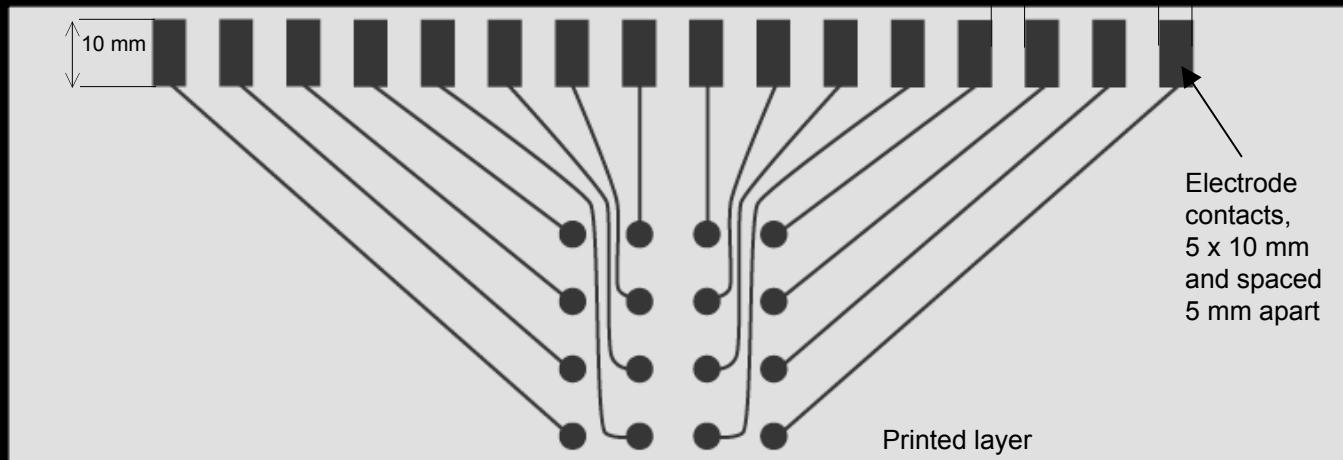
B

I, nA





**Future work: pattern SWNT forest arrays onto microchip;
collaboration with U. Edinburgh Genomics Inst. (GTI)**



Also, screen printed carbon arrays

Summary

Laser-capture microdissection techniques

Gene arrays (whole Genome)

Proteomics (MS compatible)

Biomarker Detection

Molecules involved in the pathogenesis of HNSCC

Hypothesis driven approaches (animal models)

Facilitating early detection by identifying relevant biomarkers and defining molecular targets for relevant therapeutics

Molecular Carcinogenesis

OPCB

J. Silvio Gutkind
Alfredo Molinolo
Thomas H. Bugge
Ana R. Raimondi

Expression Pathology

David Krizman
Marlene M Darfler

Proteomics

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Tim D Veenstra, NCI
Brian L Hood, NCI
Thomas P Conrads, NCI

Clinical Samples

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John Sauk, University of Maryland, MD
Nikos Nikitakis, University of Maryland, MD
Jeff Myers, MD Anderson, TX
Gary Clayman, MD Anderson, TX
Adel El-Naggar, MD Anderson, TX
David T Wong, UCLA, CA

Thank you!